



## Research Article

### BIOACTIVE WOUND DRESSINGS

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#### ABSTRACT

The research work is focused on the development of bioactive composite wound dressings with desirable wound healing properties using needle punched nonwoven, chitosan, and manuka honey. Three different methods using film, half tone and dip & dry techniques, were devised to prepare chitosan-honey wound dressings with viscose and bamboo nonwoven as secondary layer and chitosan-manuka honey as primary layer. All the three dressings prepared have been evaluated for their effectiveness as wound dressing and characterized. The results indicate that the weight and thickness of almost all the samples are comparable. The film dressings showed a higher value of air permeability and water vapor transmission of 55 cm<sup>3</sup>/cm<sup>2</sup>/second and 1700 g/m<sup>2</sup>/day. The swelling ration of film dressings was 283%. Even though dip & dry dressings showed better antimicrobial activity and higher tensile strength, their elongation is low compared to film dressings. Half tone treated samples show poor coating in the morphological observation. Hence, from the obtained results, it can be concluded that the film wound dressings show considerable advantage over half tone and dip & dry dressings.

**Keywords:** Chitosan, Dip & Dry, Half Tone, Film, Nonwoven, Manuka Honey, Wound dressings

#### INTRODUCTION

Bioactive composite wound dressing remains an active area of research in the field of medicals with a plethora of new and enhanced materials & methods. While several other researches exist in wound dressing field, the purpose of this paper is to highlight the uses of natural healing materials manuka honey and chitosan. It has been for a long time that honey is using to accelerate the wound healing.<sup>1</sup> Chitin and Chitosan were used in wound dressing treatment due to their adhesive nature, antifungal and bactericidal properties, permeability to oxygen.<sup>2,3</sup> But the usage of honey in wound healing is limited because, honey becomes a running liquid when it comes and contacts with the skin due to change in temperature.<sup>4,5</sup> Even though, several recommendations are made regarding appropriate wound dressing with honey, this research explores the possibilities of usage of manuka honey in wound dressings.

#### DRESSING PREPARATION TECHNIQUES

##### Dressing Preparation Using Film

2% (w/v) aqueous solution of chitosan was prepared in distilled water that contained 1% (w/v) lactic acid solvent. The solutions were prepared by mixing for 15 hours. Then 5 ml of 5% sodium bicarbonate was added dropwise. To the above solution 15% of glycerol and 13% of honey were added. The resultant solution was filtered, left to stand for settling down of air bubbles. Then required volume was casted onto the petri dish. Subsequently, 2cmx2cm viscose/bamboo nonwoven was placed onto the mixture. The mixtures were then dried in an oven at 40° C for 24 hours. The prepared bilayer dressings were stored at 25±1 °C and relative humidity 60-65% in an airtight glass container until further use.

##### Dressing Preparation Using Half Tone Technique

Chitosan-honey solution was well stirred and was cast onto the viscose/bamboo nonwoven fabric using the halftone technique. Each time the volume of the solution that is poured onto the nonwoven fabric was maintained as 0.05 ml/cm<sup>2</sup>.

##### Dressing Preparation Using Dip and Dry Technique

Viscose/bamboo nonwoven was immersed into the above chitosan-honey solution and frozen for 3 h. The dressing was immersed into NaOH aqueous solution for 24 h, after defrosting. Then the dressing was washed with deionized water repeatedly. The experimental plan for three types of dressings with sample codes is given in Table 1.

Table 1 Experimental plan

S.No.	Sample Code	Sample
1	FWD	Film Wound Dressing
2	HTWD	Half Tone Wound Dressing
3	DDWD	Dip & Dry Wound Dressing

#### WOUND DRESSING EVALUATION METHODS

All the three dressings were evaluated for their structural properties like air permeability, water vapor transmission rate, water absorption, tensile strength and elongation, antimicrobial activity and microbial penetration.

Mass per unit area of the nonwoven sample was measured as per the standard ASTM D3776-97. An SDL digital thickness gauge was used to measure the thickness as per ASTM D5729-97. Air permeability of the samples was measured as per the standard

ASTM D737-99. Desiccant method was used to measure the water vapor transmission rate as per the standard ASTM E96-95. The water uptake was assessed gravimetrically. Instron Universal Testing instrument was used in the measurement of tensile strength and percent elongation with a 2 kg load cell (Model 4206, Instron Ltd., Japan). The experiment was carried out as per ASTM D 882-12 standard. Antimicrobial activity against gram positive *S.aureus* and gram negative *E.coli* was assessed using the method SN 195 920.

### Effect of Dressing Preparation Technique on Structural Properties

All the dressings show uniform thickness throughout the dressing. Figure 1 shows that the mass per unit area and thickness of film wound dressings was 257GSM and 3.746mm.

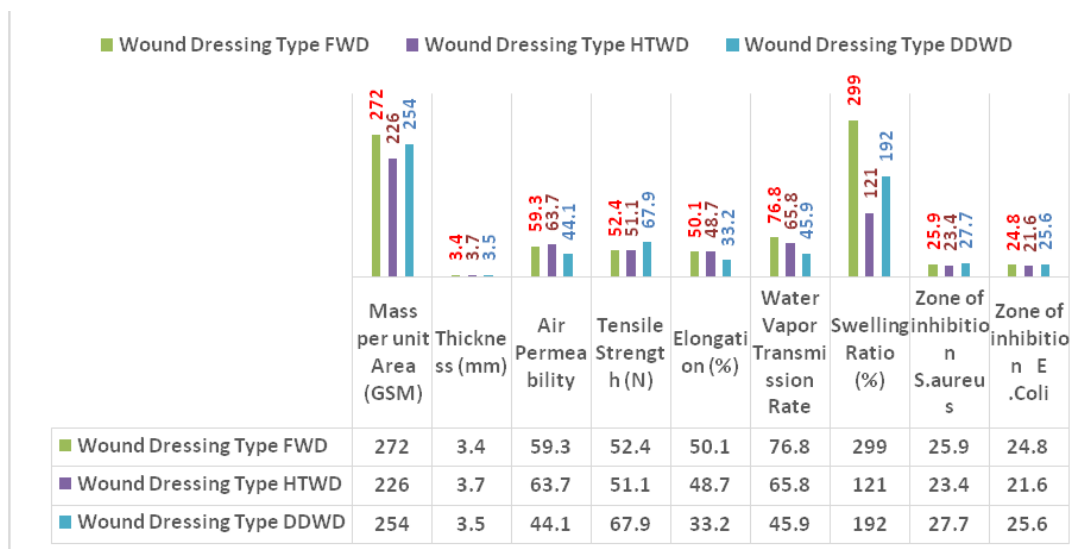


Figure 1. Structural properties of prepared wound dressings

It was found that the film dressings exhibited higher weight and thickness. Even though dip and dry wound dressing exhibited minimum thickness, the weight of the sample was higher than half tone wound dressing. Both weight and thickness influence absorbency and fluid retention capacity of the wound dressings.<sup>6</sup>

### Effect of Dressing Preparation Technique on Air Permeability of the Wound Dressings

Air permeability of a wound dressing is to be maintained to prevent the growth of anaerobic microorganisms and to control the infection of the wound due to external causes. Figure 1 shows that the air permeability values of the prepared dressings range between 36cm<sup>3</sup>/cm<sup>2</sup>/second to 58cm<sup>3</sup>/cm<sup>2</sup>/second. Air permeability values of film wound dressings show a higher value of 58cm<sup>3</sup>/cm<sup>2</sup>/second. The application of chitosan-honey solution onto the viscose/bamboo nonwoven in all the three techniques reduced the air permeability value to a notable level compared to viscose/bamboo untreated nonwoven. This might be because of the reduction in pore size in the nonwoven structure after the treatment. Half tone coated sample show the permeability value of 55cm<sup>3</sup>/cm<sup>2</sup>/second. The coating methods narrow down the holes in the structure and thus block the air in.<sup>7</sup> Since, the presence of voids in the surface of the dressings decides the air permeability of the sample the nonwoven treated with dip & dry method shows poor air permeability due to the loss of blank spaces in the surface. The significant decrement in air permeability is assigned to the dry matter content of honey and its sticking behavior<sup>8</sup>. This tends to collapse the nonwoven structure and the space between fibers in the structure, and thereby reduces the air permeability.

### Effect of Dressing Preparation Technique on Tensile Strength and Elongation

It is clear from the tensile strength and elongation results from Figure 1 that there existed an inverse relationship between tensile strength and elongation. Tensile strength and elongation of film wound dressing showed optimum results of 50.6 N and 38% respectively. Even though tensile strength of dip and dry wound dressing seems to be higher, the elongation value of the same showed a lower value. The increase in tensile strength attributed to the chitosan-honey treatment and inter-fiber friction.

### Effect of Dressing Preparation Technique on Water Vapor Transmission Rate

Wound fluid management is a great problem in injured skin, where both excessive dehydration and exudates accumulation has to be controlled to assist the healing process. The application of ideal wound dressings, maintain the water loss or accumulation from the skin at an optimal rate. Evaporative water loss from the normal skin is 700g/m<sup>2</sup>/day to 1200 g/m<sup>2</sup>/day, but for the injured skin it is in the range of 800 g/m<sup>2</sup>/day to 1300 g/m<sup>2</sup>/day. Very high water vapor permeability cause dehydration resulting in dry condition in the wound area and very low water vapor permeability accumulate wound exudates, which may cause deceleration in healing of wound and also increases the risk of bacterial growth. In this study, water vapor transmission of the samples ranged from 1000 to 1700g/m<sup>2</sup>/day (Figure 1), where the film wound dressing exhibits higher transmission of 1700 g/m<sup>2</sup>/day. This makes the film dressing more suitable to be used as wound contact layer for wound dressings. Dip and Dry wound dressings show low value of 1000 g/m<sup>2</sup>/day, which may be because, in the dip and dry dressings, the water vapor diffusivity of the fibers reduced

due to the reduction of air spaces between the fibers and reduced porosity.<sup>9</sup>

#### Effect of Dressing Preparation Technique on Swelling Ratio

To maintain a balanced moist environment, the absorption of excess exudates by wound dressing is an important factor. This property of the wound dressing prevents the air borne infection on the wound site. It is inferred from the Figure 1 that the swelling ratio of film dressings shows a highest value of 283%. Half tone and dip & dry dressings showed the swelling ration of 108 % and 178 % respectively. All the tested wound dressing samples shows good water absorption ability, which is because of hydrophilic (-OH) groups present in viscose and bamboo and also the hydrophilic nature of honey, thus they combine with water molecules chemically. In absorbent fibers, when the surface is exposed to liquid, spontaneous flow of liquid within the capillary spaces along with simultaneous diffusion of liquid into the interior of the fiber or a film on the fiber surface occurs.<sup>10</sup> The water absorption exhibits higher value of 283% for film wound dressing, which is because the film structure holds more water than the other two dressings.

#### Effect of Dressing Preparation Technique on Antibacterial Activity

Antibacterial activity of the samples against two wound infection causing bacterium (gram positive-*S.aureus* and gram negative-*E.coli*) was measured by agar diffusion method. All the samples exhibited inhibition zone against both bacteria. This is because of the fact that all the materials viscose, bamboo, chitosan and manuka honey contributes to antibacterial activity and arrests the growth of bacteria. Figure 1 shows the antibacterial activity of film dressings against *S.aureus* and *E.coli* is 24.5 mm and 24.2 mm respectively. In all the samples, the activity against *S.aureus* is significantly higher than *E.coli*. It was also noted that the inhibition zone of dressing sample coated using half tone technique against both the bacteria showed smaller values which may be because of non uniform adherence of chitosan and honey in the test area. Dip & dry treated samples show the inhibition zone of 26 mm and 24mm against *S.aureus* and *E.coli* respectively. Since the nonwoven material is completely dipped into chitosan-honey solution, the chitosan and honey content in a specified area is higher in case of dip & dry dressings, which in turn contributes to higher inhibition zone. The non-peroxide factors, low pH and osmotic effect of its high sugar content attributes to the antibacterial activity of honey.

#### Effect of Dressing Preparation Technique on Microbial Penetration Test

All the test samples were placed on top of the test tubes individually, where nutrient broth was maintained, and the control test tube was closed with a cotton ball completely against an open vial. All the test tubes were checked for their level of contamination by visual observation. The open vial was totally contaminated due to the environmental bacteria in contrast with the clear cotton ball closed vial without any contamination. In the three of the tested samples, the film sample and dip & dry sample exhibited similar result to that of the control sample which shows the higher inhibition for microbial penetration. The half tone coated sample has been contaminated significantly compared to the other two. The level of contamination is negligible compared to the open vial.

#### Surface Morphological Studies by Scanning Electron Microscope

SEM pictures of film wound dressing (Figure 2) shows plain surface with scales like fibrous structure. Obvious pores were not observed, but this fibrous structure allows the film to absorb more wound fluids effectively thereby keeping the microbes out of wound bed.

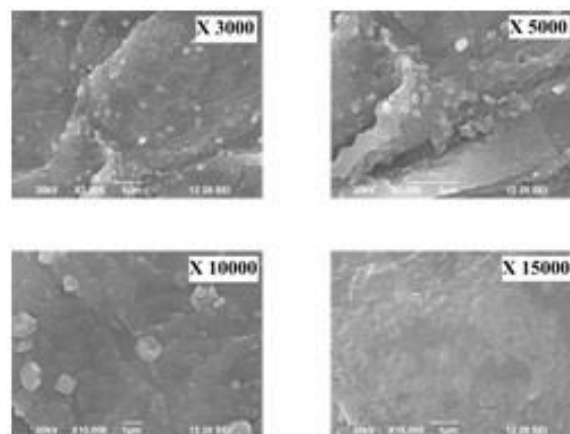


Figure 2 SEM photographs of outer surfaces of FWD at different magnifications

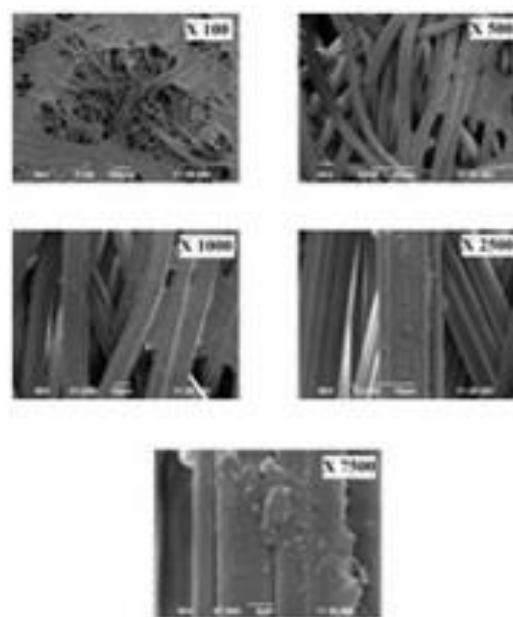
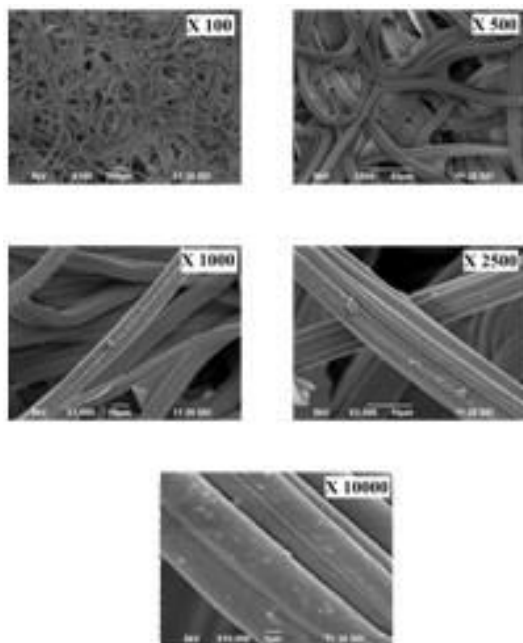


Figure 3 SEM photographs of outer surfaces of HTWD at different magnifications

Half tone treated sample shows more cracks on the surface, where lot of junction zones and pin holes were created during the coating. This reduces the strength of the sample to a notable extent. Higher magnifications show the coating of the chitosan honey solution on the fiber surface (Figure 3).

Dip and dry treated sample shows a compact surface with good structural integrity due to fact that the chitosan-honey solution blocked the void area of the nonwoven. Hence, there exists a good interfacial adhesion between the fibers and solution due to the stickiness of the nature of honey (Figure 4).



**Figure 4 SEM photographs of outer surfaces of DDWD at different magnifications**

## CONCLUSION

The mass per unit area and thickness of film wound dressings was 257 GSM and 3.746mm. It was found that the film dressings exhibited higher weight and thickness. The air permeability values of the prepared dressings range between 36cm<sup>3</sup>/cm<sup>2</sup>/second to 58cm<sup>3</sup>/cm<sup>2</sup>/second. Air permeability values of film wound dressings show a higher value of 58cm<sup>3</sup>/cm<sup>2</sup>/second. Tensile strength and elongation of film wound dressing showed optimum results of 50.6 N and 38% respectively. Water vapor transmission of the samples ranged from 1000 to 1700g/m<sup>2</sup>/day, where the film wound dressing exhibited higher transmission of 1700 g/m<sup>2</sup>/day. Dip and dry wound dressings showed low value of 1000 g/m<sup>2</sup>/day. The swelling ratio of film dressings showed a highest value of 283%. Half tone and dip & dry dressings showed the swelling ratio of 108 % and 178 % respectively. The antibacterial activity of film dressings against *S.aureus* is 24.5mm and *E.coli* is 24.2mm. Dip and dry treated samples showed higher inhibition zone of 26.2 mm and 24.4 mm against *S.aureus* and *E.coli* respectively. In microbial penetration test, the film sample and dip & dry sample exhibited clear results (no contamination of nutrient broth), which is similar to that of the control sample. This shows that the higher inhibition for microbial penetration in case of both film and dip & dry dressings. In summary, 70/30 viscose-bamboo needle punched nonwoven fabrics treated with

chitosan-honey solution with three different methods were compared with specific reference to wound dressings. Hence, from the obtained results, it can be concluded that the film wound dressings show considerable advantage over half tone and dip & dry dressings.

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